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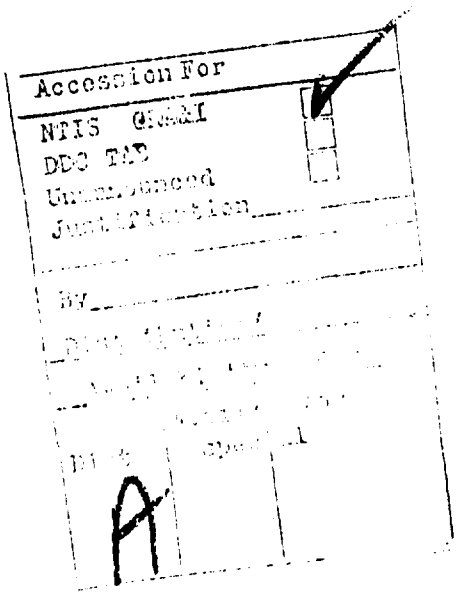

(6) RIBAVIRIN TREATMENT OF TOGA-, ARENA- AND BUNYAVIRUS INFECTIONS IN SUBHUMAN PRIMATES AND OTHER LABORATORY ANIMAL SPECIES

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I. SUMMARY

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II. INTRODUCTION

Antiviral chemotherapy offers an approach to the control of certain viral diseases which are not amenable to control by vaccine prophylaxis. The development of animal models to represent virus infections of humans has resulted in many useful avenues for investigating the effectiveness of poten-

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II. INTRODUCTION

Antiviral chemotherapy offers an approach to the control of certain viral diseases which are not amenable to control by vaccine prophylaxis. The development of animal models to represent virus infections of humans has resulted in many useful avenues for investigating the effectiveness of poten-

tial antiviral agents, and for exploring both the requirements and limitations of successful treatment. In our institute, we are concerned with a number of diseases which would be considered "exotic" in the United States, but which are of considerable global significance. Examples of representative "model" virus infections include yellow fever (YF) in rhesus monkeys (a flavivirus infection; Hilmas et al., 1976), Venezuelan equine encephalomyelitis (VEE) in mice and monkeys (an alphavirus infection; Kuehne et al., 1977; Stephen et al., 1979) and Machupo (MAC) infection in rhesus monkeys (an arenavirus; Eddy et al., 1975).

Preliminary evaluation of potential antiviral compounds is usually done using VEE, YF, Rift Valley fever (RVF, a bunyavirus) and Pichinde (PIC, an arenavirus) viruses in mice, guinea pigs or hamsters. Compounds found to be active against these viruses are evaluated further to establish optimum dose schedules, toxicity and therapeutic potential. Efficacy, as determined by studies in rodent species, is then established further in subhuman primate models. Viruses are usually low passage and are chosen to mimic the human disease as closely as possible with respect to patterns of infection and sites of replication. Thus, it can be assumed that compounds shown to have antiviral activity in these primate models may similarly be shown to have efficacy when evaluated in man.

III. RESULTS

A. Cell Dependence of Antiviral Activity

We have begun to compare the action of ribavirin *in vitro* using selected cell lines as analogs of differentiated cell types *in vivo*. Our data are too scanty to permit any generalizations, but nevertheless demonstrate important differences and confirm the expected complexity of the situation. Figure 1 contrasts the antiviral effect of ribavirin on the yield of RVF virus from a mouse macrophage cell line (BW-JM) and Vero cells, a monkey kidney cell line. Yield of virus from BW-JM cells was significantly lower ($P < 0.001$) at all drug concentrations tested, whereas only the highest concentration (100 ug/ml) was effective in Vero cells. When a murine glial cell line was compared to Vero cells, low concentrations (25 ug/ml) of ribavirin were markedly inhibitory to RVF virus replication; however, classical L cells responded similarly to Vero cells. Thus, the differences are not solely due to the species of origin. Furthermore, at least some nervous tissues are sensitive to the antiviral activity of the drug.

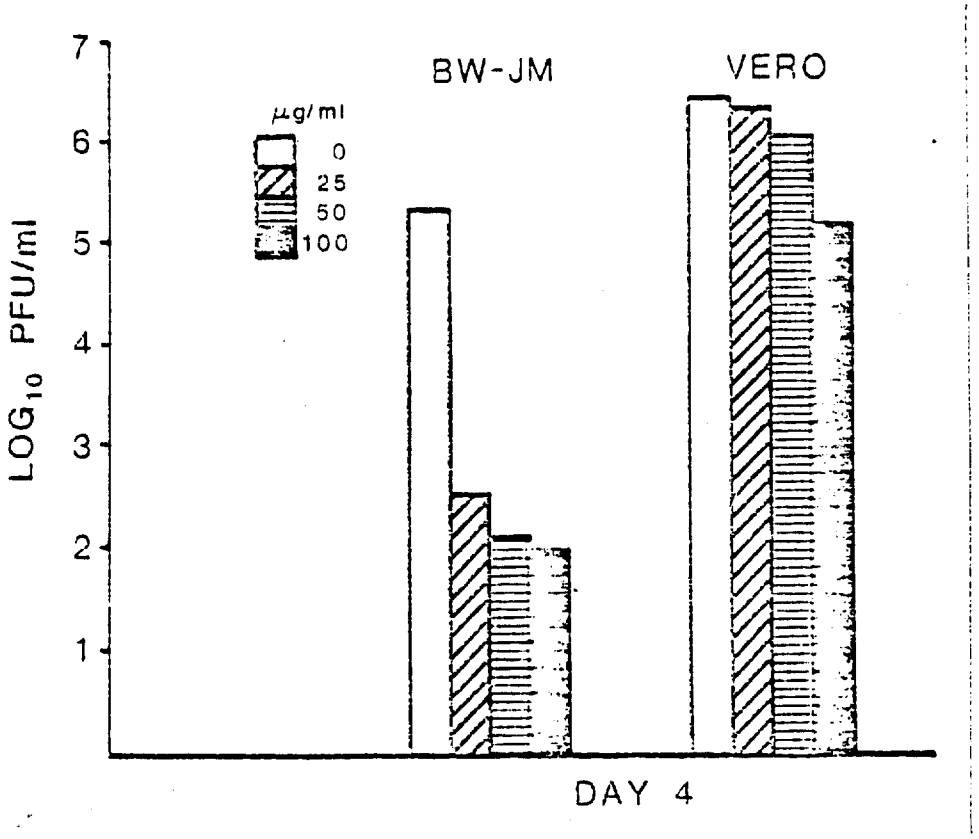

FIGURE 1. In vitro activity of ribavirin against RVF in BW-JM and Vero cells.

Preliminary experiments revealed striking differences between the metabolism of ^{14}C-labeled ribavirin in these cell lines. BW-JM cells accumulate radioactivity rapidly, whereas Vero cells do not reach a steady-state until 48 hours after the addition of labeled ribavirin to the medium. Following a washout procedure using maintenance media free of ribavirin, the trend reversed. After 1 hour, only 15 percent of the labeled ribavirin was retained by BW-JM cells and only 1 percent at 24 hours. Glial and Vero cells retained approximately the same amount of radioactivity at 1 hour (62 percent vs. 69 percent, respectively), but counts were lower in glial cells (27 percent) than Vero cells (51 percent) at 6 hours. Consequently, in addition to specific factors of virus replication that affect drug activity, the ability of cells to absorb, metabolize and retain drug appears to be an important determinant of activity.

B. Drug Dose as a Function of Antiviral Activity

Mice were infected with RVF virus (200 PFU, s.c.) and treated with various doses of ribavirin (Figure 2). Deaths in

untreated mice were the result of fulminant hepatitis. Occasional untreated mice survived the acute hepatitis, but died of later encephalitis. When the dose of ribavirin was 20 mg/kg or higher, survival was markedly increased with 100 percent survival occurring in mice treated with 100 mg/kg/day. Treated mice that died at doses below 100 mg/kg, died of the late-occurring encephalitis. Doses of ribavirin higher than 100 mg/kg were toxic.

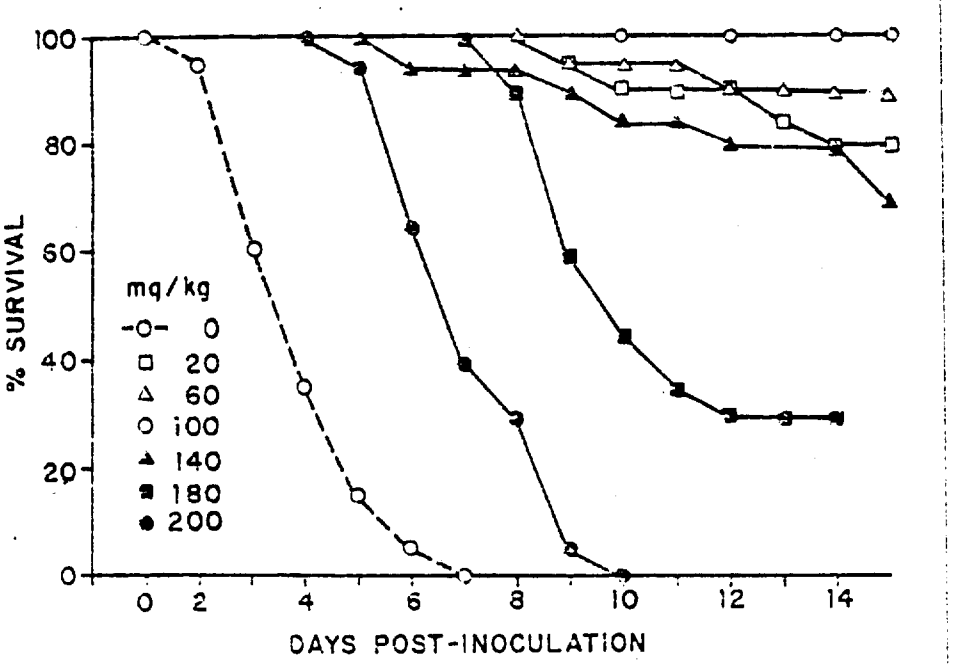

FIGURE 2. Survival of RVF-infected $C_{57}Bl_6$ mice treated with varying doses of ribavirin (n = 20/group initially).

C. Drug Distribution as a Function of Antiviral Activity

Failure of ribavirin to prevent death in mice challenged with viruses that cause encephalitis, in spite of clearly demonstrable in vitro antiviral activity, suggested that ribavirin did not penetrate the central nervous system. Rift Valley fever virus was inoculated either intraperitoneally (100 PFU, i.p.) or intracranially (1 PFU, i.c.) into mice given ribavirin by the i.p. route (Figure 3). Ribavirin significantly increased ($P < 0.01$) the number of survivors and the time to death in mice challenged i.p., but not i.c. These data appear to support the supposition that ribavirin is not effective against encephalitis viruses because of its failure to penetrate the blood-brain barrier. Pharmacokinetic data

presented elsewhere (Dr. D. Catlin, this volume) further clarify these observations.

D. Time of Initial Treatment in Relation to Pathogenesis

Pichinde is not a high-hazard virus in man. This virus can be adapted so that MHA hamsters and strain 13 guinea pigs die following s.c. challenge. MHA hamsters inoculated with adapted PIC virus (10^4 PFU, s.c.) began to die by day 8 (Table 1). In contrast, hamsters treated with ribavirin on days 0 through 14 did not die. Treatment delayed until day 4 after virus inoculation delayed the time to death and increased the number of survivors in comparison to the untreated group. Further elucidation of the infection showed that virus was detectable in blood by day 3 and reached a mean peak viremia of approximately 10^7 PFU/ml by day 6 in untreated hamsters (Table 2). No viremia was detected in hamsters treated from day 0; peak viremia was lower in hamsters treated initially on day 4. Virus concentration was assayed in various tissues. Interestingly, virus was detected in the spleen and other tissues of hamsters treated from day 0, even though no virus was detected in serum (Table 2). In addition, virus was detected in spleens of hamsters (group treated on days 0-14) on day 18, four days after the cessation of treatment.

E. Studies in Subhuman Primates (Intramuscular Administration of Ribavirin)

Rhesus monkeys inoculated with RVF virus did not die. Sham-treated control monkeys were detectably viremic one day after virus inoculation and reached peak viremia titers of nearly 10^5 PFU/ml by day 2 (Figure 4). Ribavirin treatment was initiated 2 hours after inoculation of virus and was continued at 8-hour intervals. The viremia was significantly ($P < 0.001$) less in treated monkeys compared to values for control monkeys.

The pathogenesis of Bolivian hemorrhagic fever (BHF) caused by MAC virus in rhesus monkeys has been reported previously (Eddy et al., 1975). Briefly, an acute hemorrhagic phase occurred that coincided with the increase in viremia. Approximately 80 percent of untreated monkeys died during this acute phase. Of the remaining 20 percent, most developed a late neurological syndrome and died between days 20 and 40 postinfection. BHF infection in man correlates with the acute phase in monkeys (Eddy et al., 1975). Human patients that survive the acute hemorrhagic disease do not subsequently die of the late neurological phase seen in monkeys (Oldstone and

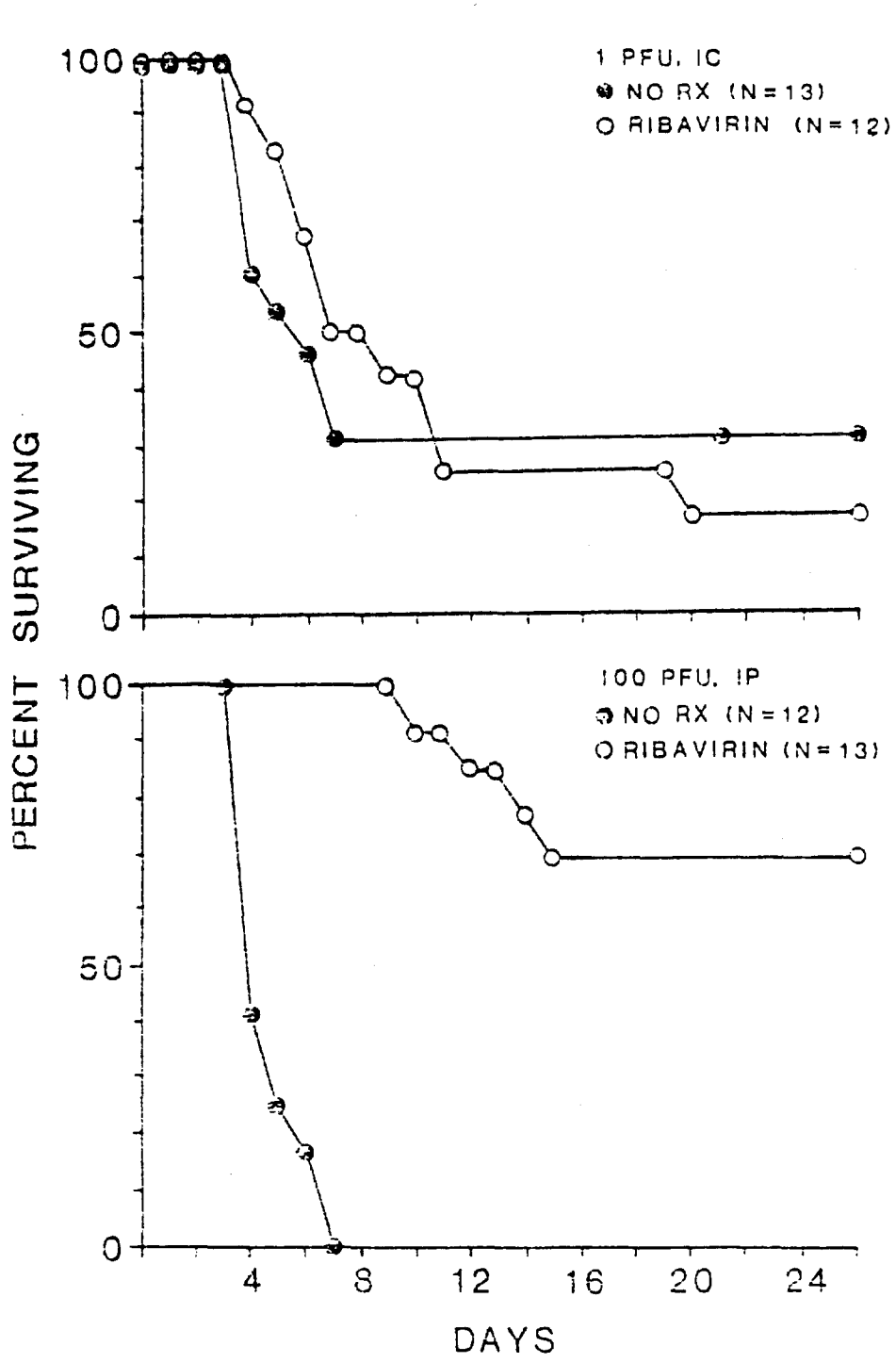

FIGURE 3. Effect of ribavirin in mice challenged with RVF virus either i.p. or i.c.

TABLE I. Effect of Ribavirin on Survival of MHA Hamsters Inoculated with PIC Virus (10,000 PFU, s.c.).

Days after inoculation	% Survival		
	Virus only (n=27)	Ribavirin (15 mg/kg, s.c., 2X daily) Days 0-14 (n=20)	Days 4-14 (n=10)
7	100	100	100
8	95	100	100
9	60	100	100
10	54	100	100
11	38	100	90
12	34	100	60
13	25	100	50
14	20	100	50
15	11	100	50
21	11	100	50

TABLE II. Comparison of PIC Virus Concentrations of Ribavirin-Treated (see Table I) and Untreated MHA Hamsters (n = 3).

Day	Log_{10} PFU/ml or g ± SE		
	No treatment	Ribavirin Days 0-14	Ribavirin Days 4-14
Plasma			
3	3.3 ± 0.86	< 0.7 ± 0	NT*
6	7.5 ± 0.10	< 0.7 ± 0	5.2 ± 0.18
9	6.8 ± 1.40	< 0.7 ± 0	3.8 ± 1.76
12	6.2 ± 0.15	< 0.7 ± 0	4.9 ± 0.43
15	5.3 ± 0.30	< 0.7 ± 0	3.9 ± 0.40
18	Died	< 0.7 ± 0	< 0.7 ± 0
Spleen			
3	6.0 ± 1.0	5.2 ± 1.0	NT
6	8.7 ± 0.20	0.6 ± 0.26	6.7 ± 0.20
9	6.8 ± 0.40	2.9 ± 1.93	5.6 ± 1.12
12	7.1 ± 0.14	3.1 ± 1.10	5.8 ± 0.15
15	6.4 ± 0.17	< 0.7 ± 0	5.1 ± 0.33
18	Died	2.2 ± 0.17	3.4 ± 0.03

*Not tested.

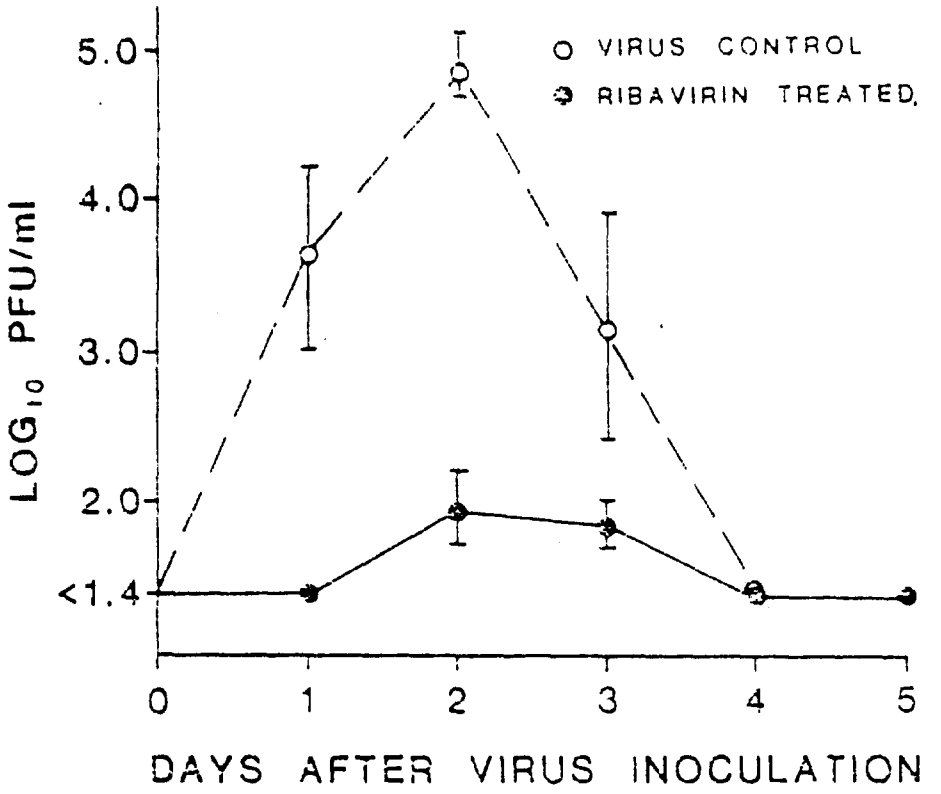

FIGURE 4. Viremia response of rhesus monkeys infected with RVF virus and treated with ribavirin (n = 4/group).

Peters, 1978).

The virological events during BHF infection of monkeys resemble those of PIC infection of MHA hamsters. Sham-treated monkeys were detectably viremic by day 5 after virus inoculation and reached peak viremia titers by day 15, after which deaths began to occur (Figure 5). Ribavirin triacetate treatment was initiated on day 0, at the time of virus inoculation and continued twice daily through day 17. Monkeys given 10 mg/kg/dose had a diminished viremia response when compared to controls, and survived through day 35. When the dose was increased to 20 mg/kg/dose, the viremia was delayed in onset and returned to undetectable titers by day 11 or 12.

We did a second study in BHF-infected rhesus monkeys using the parent compound, ribavirin. Ribavirin and ribavirin triacetate had been shown to be of similar potency using MAC virus-infected guinea pigs. Again sham-treated virus control monkeys reached peak viremia by days 12 to 14, after which they began to die (Figure 6). Monkeys treated with ribavirin developed fever by days 4 to 7 after virus inoculation. Treatment of individual monkeys was initiated at the time of onset of fever and was continued every 8 hours for 10 days.

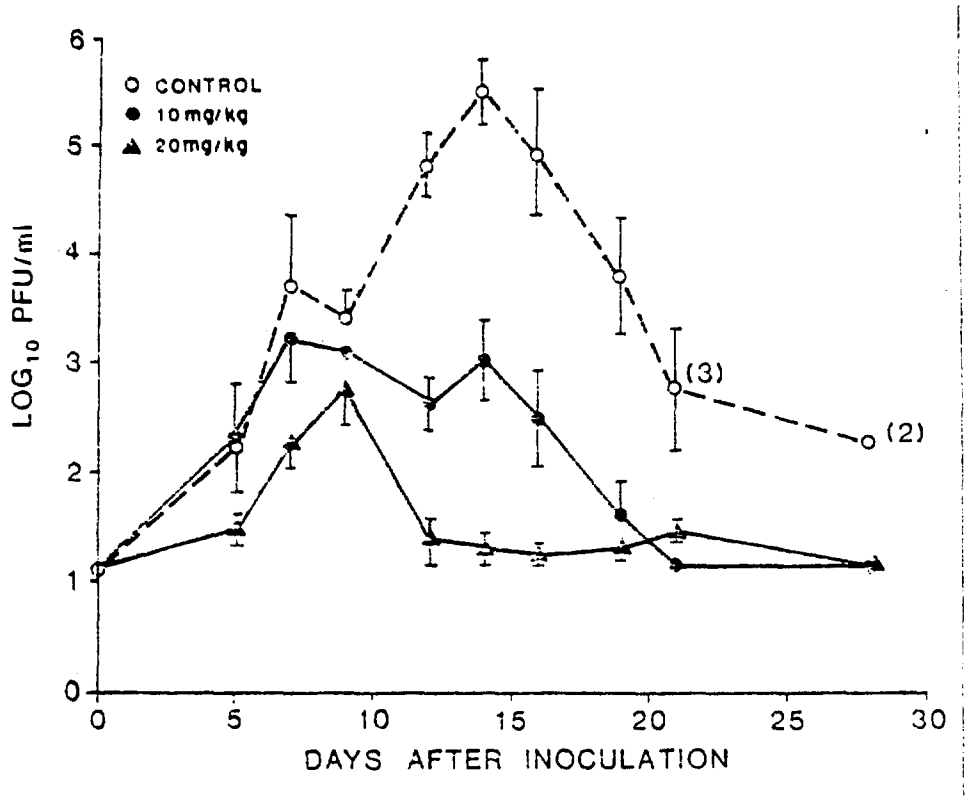

FIGURE 5. Effect of triacetylribavirin in MAC virus-infected rhesus monkeys. Treatment was given twice daily on days 0 through 17. (n = 4/group, initially; parentheses indicate altered group n).

Quite remarkably, viremia responses of treated monkeys were lower by day 7 compared to sham-treated control monkeys and virtually undetectable by day 10. As in the previous study, treated monkeys survived the acute hemorrhagic phase. A third experiment was done to confirm the results of this experiment to evaluate therapeutic potential. The results of these trials are shown in Table III. In all three studies, regardless of the time of initial treatment or regimen, ribavirin (or its triacetate) prevented death during the acute phase of illness. The late neurological syndrome, seen in 20 percent of infected, untreated control monkeys, was not prevented.

Finally, unadapted Lassa virus (10,000 PFU, s.c.) was used to infect rhesus monkeys (Jahrling et al., in press). Virus replication was similar to PIC infection in MHA hamsters and MAC infection of rhesus monkeys (Figure 7). The overall mortality in infected, untreated monkeys was 60 percent. Viremia titers continued to increase in monkeys that eventually died, whereas surviving infected control monkeys had a diminished rise in viremia. Ribavirin treatment (50 mg/kg, loading dose; 10 mg/kg, three times daily through day 18) initiated on day 0

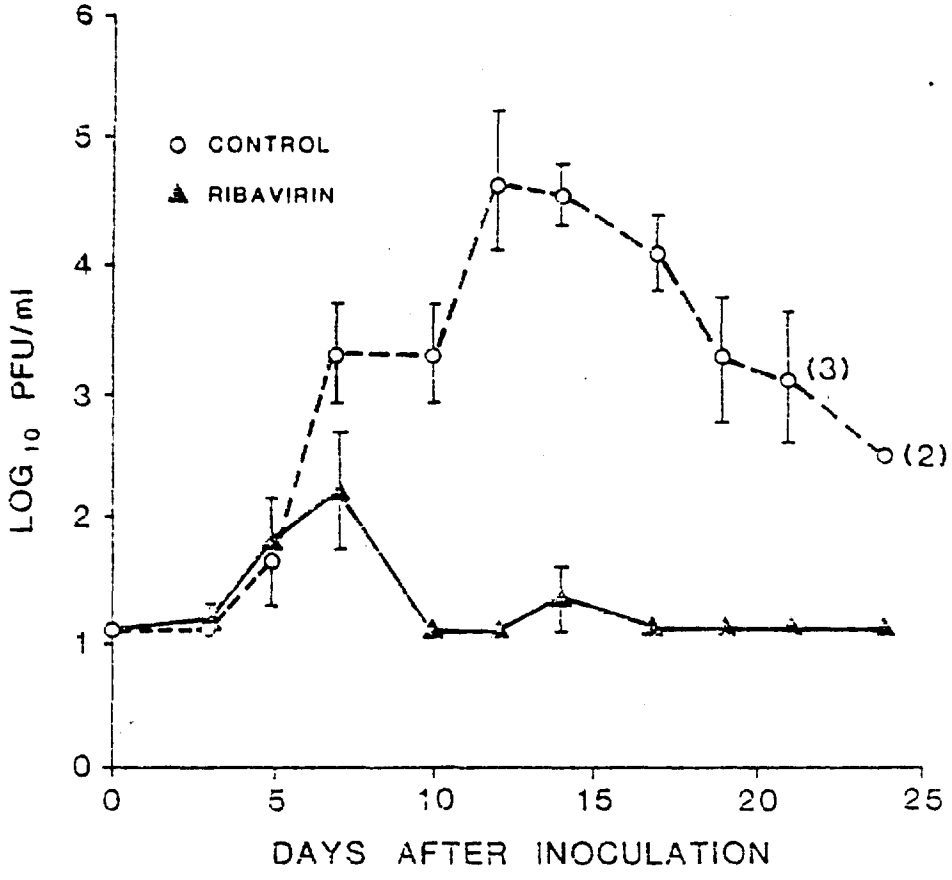

FIGURE 6. Effect of ribavirin given therapeutically to MAC virus-infected rhesus monkeys. Treatment was initiated at the onset of fever and continued three times daily for 10 days. (n = 4/group, initially; parentheses indicate altered group n).

caused a delay in onset of viremia; all treated monkeys survived (Figure 8). When initial treatment was delayed until day 5, the viremia response was similar to that of surviving controls. As in the early-treatment group, all treated monkeys survived. Both groups of treated monkeys had significantly ($P < 0.01$) lower SGOT and SGPT values, compared to infected control monkeys (Figure 9).

TABLE III. Effect of Treatment on Survival and Time to Death of MAC Virus-Infected Monkeys (n = 4/group).

Study	Group	% Survival Day 35	% Survival Day 90	MTD (Days ± SE)
1	Saline	0	0	26 ± 3
	Ribavirin triacetate			
	10 mg/kg/injection	100	25	71 ± 17*
	20 mg/kg/injection	100	0	63 ± 15*
2	Saline	0	0	23 ± 4
	Ribavirin	100	25	43 ± 6
3	Saline	25	0	36 ± 21*
	Ribavirin	100	25	39 ± 2*

*Sacrificed when paralyzed.

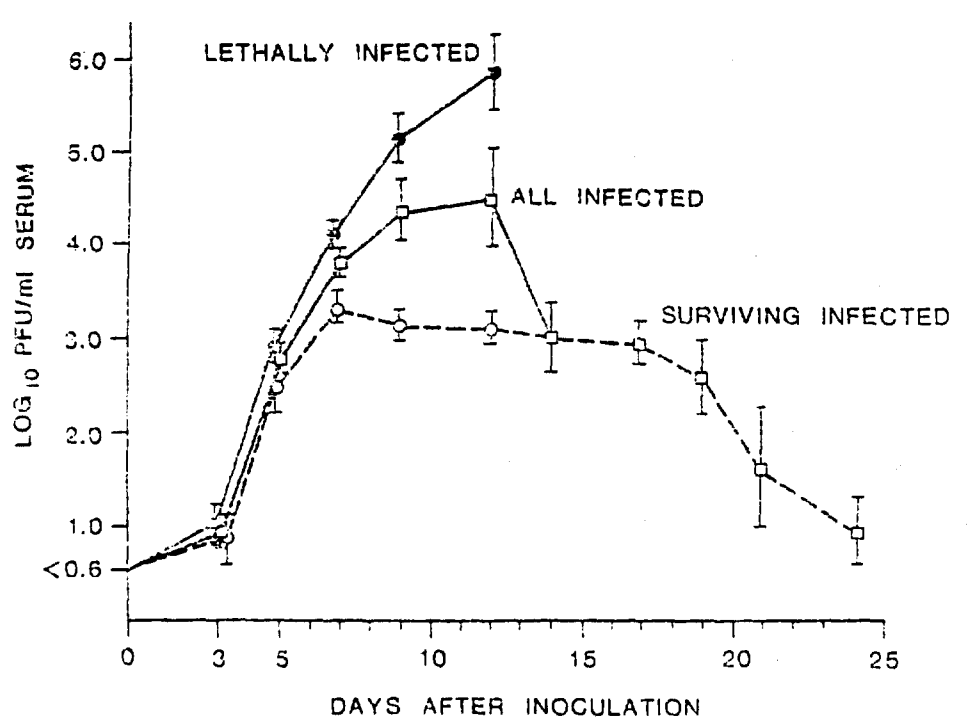

FIGURE 7. Viremia response of rhesus monkeys inoculated with Lassa virus (n = 4/group).

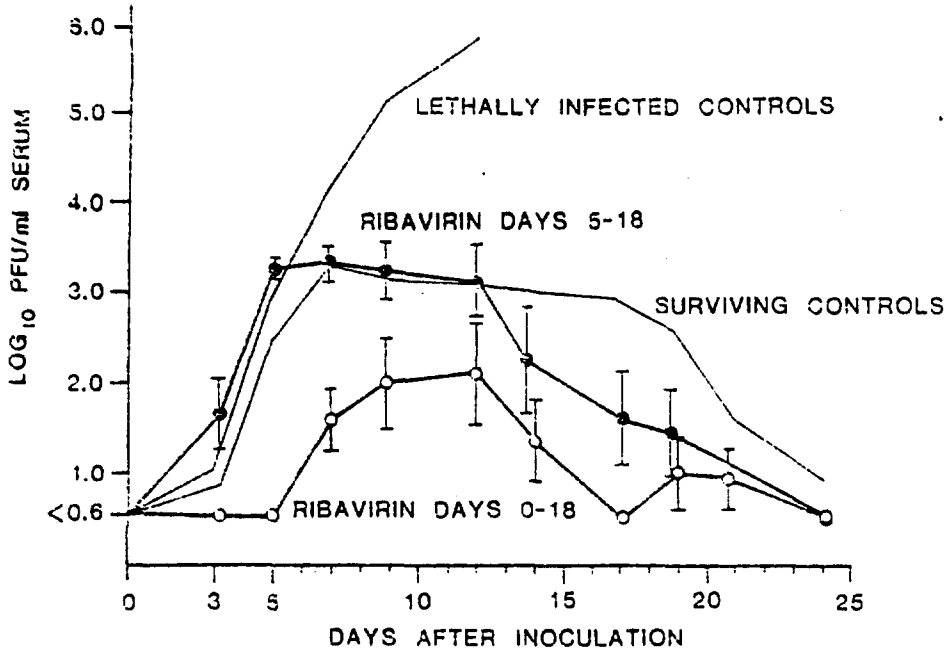

FIGURE 8. Effect of ribavirin treatment on the viremia response of Lassa virus-infected rhesus monkeys (n = 4/group).

IV. DISCUSSION

Ribavirin was effective in the treatment of RVF, MAC and Lassa virus infections in rhesus monkeys. An overall summary of the effects of ribavirin against various RNA viruses is shown in Table IV. *In vitro* data failed to correlate with *in vivo* experiments, especially in the alpha- and flavivirus groups. The reasons for this lack of correlation are not clear, but in the instances where encephalitis is the cause of death, one possibility is that inadequate drug concentrations are obtained in the CNS during ribavirin treatment. Another important observation from the various experiments is that treatment should be continued until the host's immunological system can respond specifically to the infection. An earlier report on the activity of ribavirin *in vitro* showed that the drug must remain in the system to maintain effective antiviral activity, since ribavirin is not virucidal (Huffman *et al.*, 1973).

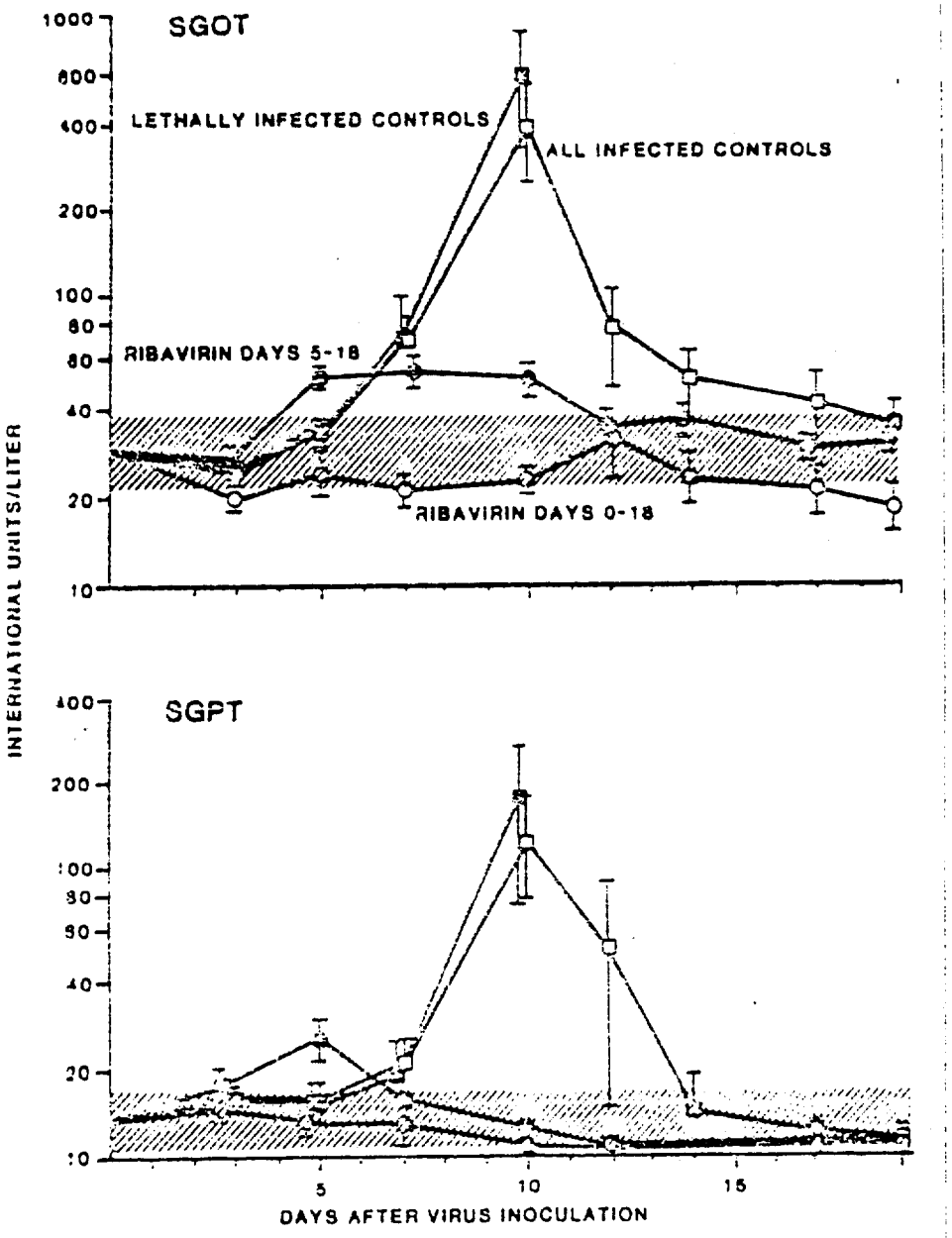

FIGURE 9. Effect of ribavirin treatment on serum transaminase levels in Lassa virus-infected rhesus monkeys (n = 4/group).

TABLE IV. Antiviral Activity of Ribavirin in Various Test Systems.

Virus Group	Ident.	In vitro* 5-25 µg	In vitro* 100 µg	In vivo Rodents	In vivo Monkeys
Alpha-	VEE	+	±	- (mice)	Not done
	CHIK	+		No model	-
Flavi-	JE	+		- (mice)	Not done
	YF	+		- (mice)	±
	DEN-1	+		No model	Not done
	DEN-2	+		No model	Not done
Arena-	PIC	+		+ (MHA hamsters) + (guinea pigs)	No model
	MAC	+		+ (guinea pigs)	+
	LAS	+		+ (guinea pigs)	+
Bunya-	RVF	+	+	+ (mice)	+
	SFS	+		No model	No model
Myxo-	Influenza			+ (mice)	+

* Ribavirin concentration in µg/ml.

Preliminary experiments using PIC and Lassa viruses have shown the sites of preferential virus replication to be strikingly similar to the tissue distribution of ^{14}C-labeled ribavirin. Since RVF virus infection is associated with acute hepatitis, the syndrome associated with this virus also is quite compatible with an effective action of ribavirin, based on its tissue distribution. It is conceivable that developing pharmacokinetic data will make it possible to refine the dose and regimen of therapy to achieve the maximum effect. Further, the possibility of combined therapy exists, utilizing a second antiviral drug or specific immune plasma.

The ultimate value of ribavirin in the treatment of potentially fatal virus infections of man remains to be evaluated. The data presented here serve to assist in the development of effective therapeutic approaches to these important, as-yet uncontrolled virus infections of man.

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